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Growth and Enterotoxin A Production by *Staphylococcus aureus* in Precooked Bacon in the Intermediate Moisture Range

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ABSTRACT

Different water activities were obtained in precooked bacon by varying the frying time. Water activity (a_w) correlated best to the moisture, salt and protein content. When stored aerobically at 37°C, *S. aureus* A100 was capable of rapid growth in precooked bacon at a a_w of 0.84 or above, whereas at 20°C a a_w of 0.88 or higher was required. Under anaerobic storage at 37°C, growth was observed at a a_w of 0.90, and at 20°C slight growth was noted at a a_w of 0.91. The increase in the minimal a_w required for aerobic growth at the lower temperature was reflected in the differences between the isotherms obtained at 37°C and 20°C. The maximum populations achieved were higher for samples stored aerobically. Enterotoxin A (19-821 ng/g) was found in all aerobically stored samples where growth occurred. Enterotoxin A (38-109 ng/g) was also found in all anaerobically incubated samples where the population of *S. aureus* increased more than one logarithmic cycle.

INTRODUCTION

Water activity (a_w) has been widely recommended as a parameter to control microbial growth in intermediate moisture foods (Scott, 1957; Troller, 1976), which normally range in a_w from 0.7–0.9 and in water content from 20–50% (Karel, 1973). Among food-borne bacteria, *S. aureus* is unique in its ability to survive, grow, and produce enterotoxins in an environment with low a_w (Lotter and Leistner, 1978). While *S. aureus* is normally not a good competitor in high moisture foods containing a mixed microflora, it can be in a low a_w environment where the growth of most of the other competitive microorganisms is either prohibited or restricted. Usually, the growth of mold and yeasts, which are more resistant to lower a_w's than bacteria, are controlled in intermediate moisture foods by the addition of antimycotic agents such as potassium sorbate or by using vacuum packaging.

Troller and Christian (1978) have reviewed the problem of *S. aureus* in cured meats and noted its ability to grow in the presence of salt, $NaNO_3$ and $NaNO_2$ and, under certain conditions, to produce enterotoxins.

Precooked bacon is an intermediate moisture food and a ration item currently being used by the military. For this product, military specifications (U.S. Department of Defense, 1974) require a mean moisture to salt ratio (percent moisture, M, divided by percent salt, S) of 9 or less to help assure the safety of vacuum packed, precooked canned bacon which is to be stored without refrigeration. This requirement was based on the study of Whiting et al. (1964), who found that, for precooked bacon, an M/S ratio of 9 corresponded to an index of M/S times the reciprocal of protein (P) concentration (M/SP) of 0.4 and that this index corresponded to a a_w of 0.9, a value, reported by Scott (1953), that was capable of preventing the anaerobic growth of *S. aureus*. For routine analysis it is considerably easier to use the M/S ratio in a quality control program rather than M/SP. Powers et al. (1978) though, analyzed precooked, canned bacon produced for the U.S. Department of Defense and rejected because of inadequate processing. *Staphylococcus aureus* was found in 9% of the 221 samples analyzed and all of the contaminated samples had an M/S ratio of 8.73 or higher and a a_w range of 0.86–0.96 indicating that the relationships between M, S, P and a_w suggested by Whiting et al. (1964) may require additional verification.

This study was therefore undertaken to ascertain the ability of a_w, temperature and oxygen to modify growth and enterotoxin secretion of *S. aureus* in prefried bacon and to verify the validity of using an M/S ratio of 9 for preventing microbial growth.

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MATERIALS & METHODS

Preparation of precooked bacon

Bacon slabs were purchased from a local meat packer, tempered at −2.2°C (28°F) overnight, and sliced to a thickness of 2 mm. To minimize variation between samples, which was due to heterogeneity in the distribution of th constituents in the bacon slabs, a grouping procedure was employed in which each of four consecutive slices were randomly assigned to one of four frying times. The bacon slices were placed in the bottom of a wire basket without overlapping and held down with an aluminum grid and fried at 177°C (350°F) in a deep fat fryer for the assigned time interval. The cooked bacon slices were drained and lightly patted with a paper towel to remove excess oil. After cooling, a Waring Blendor was used to reduce the size of the fried bacon slices to coarse particles. The bacon particles were then subjected to a_w measurements, chemical analysis, and inoculation studies. Data from a_w measurements and chemical analysis indicated that the use of randomized blocks reduced variation between bacon samples to an acceptable level.

Water activity measurement

A water bath capable of precise temperature control (± 0.02°C, Greenspan, 1975) was used for both the measurement of a_w and the incubation studies. The a_w measurement unit (Fig. 1) consisted of a glass humidity chamber (9) [1/2 pt Mason jar, Ball Corp., Muncie, IN] and a sensor assembly. The sensory assembly included a stainless steel lid (10), a probe (2) and a narrow range hydrosensor (7) [Model 4-4820, 4-4821, or 4-4822, American Instrument Co., Spring, MD]. The probe (2) consisted of a hollow stainless steel tube, which had a sensory receptor at the lower end and which was wired through the tube to a cable connector (1) at the upper end. Signals from the sensory assembly were read on a Digital II Hygrometer Indicator (Model 15-3057, American Instrument Co., Silver Spring, MD). The probe was secured to the lid by a threaded ring (3). Tightening the threaded ring compresses the rubber grommet (4) between the lid and the probe, making the seal leak-proof. The sensory assembly was then held against the glass container by means of a stainless steel cap (5), which was screwed against the lid by engaging the threads of the glass container with internal threads in the cap. A gasket (6) was placed on the outer edge of the lid to prevent leaks.

In measuring a_w, 50g of ground fried bacon (8) were placed in the container, the unit sealed and then submerged in the water bath for 6–8 hr for temperature and a_w equilibration. After equilibration, the reading was taken with the Digital II Hygrometer Indicator. A calibration curve between the Indicator readings and a_w was used for determining the actual a_w's of the samples. Each individual calibration curve was constructed by plotting the readings of the sensor for known saturated salt solutions vs the respective theore-

tical a_w's of the saturated salt solutions at the same temperature (Greenspan, 1977). The sensors were calibrated at least once a month.

Chemical analysis

Procedures of the Association of Official Analytical Chemists (AOAC, 1975) were used for the determination of protein, fat, salt, moisture and ash constituents.

Inoculations study

Unless otherwise indicated, *Staphylococcus aureus* A-100, an enterotoxin A (SEA) producing strain was used throughout this study. The culture was maintained on slants of trypticase soy agar supplemented with 0.5% yeast extract (TSY) and transferred weekly. The medium used in the inoculum preparation contained 3% Protein Hydrolysate Powder (Mead-Johnson, Evansville, IN) and 3% NZAmine-NAK (Sheffield Chemical, Norwich, NY) supplemented with filter-sterilized nicotinic acid (0.001%) and thiamin (0.0005%). The inoculum was prepared by incubating the inoculated medium (100 ml in a 500 ml Erlenmeyer flask) at 37°C for 16 hr on a gyrotary shaker (Model G-2, New Brunswick Scientific Co., New Brunswick, NJ) at 280 rpm. The culture was harvested, centrifuged, and washed three times with 0.1M phosphate buffered saline, pH 7.2 (PBS), and then resuspended in PBS.

The washed cells were further diluted with Butterfield's phosphate buffer (APHA, 1972) so that the cell population in the inoculated bacon was approximately 10^5–10^6 CFU/g. A Hobart processor (Model N-50, Hobart Manufacturing Co., Troy, OH) was used for distributing the inoculation in the bacon, by mixing 1 ml of an appropriately diluted cell suspension dropwise,into 500g of ground bacon. A preliminary study had demonstrated that this technique resulted in the homogeneous distribution of cells and that the addition of the cell suspension did not significantly alter the a_w of the bacon.

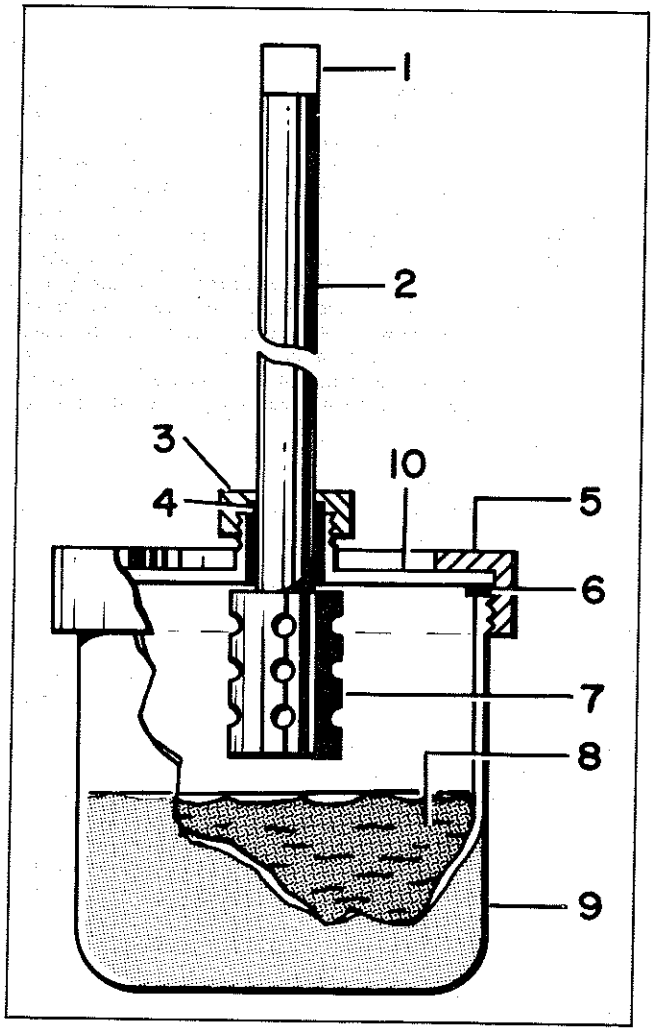

Fig. 1—Sensor assembly and humidity chamber for measuring a_w: (1) connector to the digital readout; (2) stainless steel tube with internal wiring; (3) threaded ring; (4) rubber compression grommet; (5) threaded ring cap; (6) viton gasket; (7) sensor; (8) food sample or saturated salt solution; (9) glass Mason jar; (10) stainless steel lid.

For growth studies of inoculated bacon maintained at specific a_w's a 1-pint mayonnaise jar (Andler Bottle Co., Cambridge, MA) was used as the humidity chamber and the sensor assembly plate was replaced by a flat plate. For aerobic studies, after inoculation, ten gram portions of bacon were distributed in the bottom half of sterile petri dishes (60 X 15 mm, Falcon brand, Becton-Dickinson and Co., Cockeyville, MD). Two petri dishes were then suspended in each humidity chamber on stainless steel shelves supported from the lid by chains. The lid with its assembly was then secured, as in Figure 1, to the glass container with a threaded cap and a washer. When appropriate, a saturated salt solution was placed on the bottom of the chamber. The humidity chamber was then submerged in the water bath during incubation.

For anaerobic studies 40g of inoculated, ground bacon was placed in sterile 211 X 101.5 cans, vacuum (21 inches) sealed (Model 722, Rooney Machine Co., Bellingham, WA), and incubated at the desired temperature. The vacuum inside the cans was determined with a Budenberg Vacuum Gauge (Broadheath, N. Manchester, England) immediately prior to analysis. The canning procedure did not alter the a_w of the bacon samples.

Microbiological analysis

The homogenates of the bacon samples were prepared for analysis by stomaching (Model 400, A.J. Seward and Co. Ltd, London, England) 10g samples with 90 ml of 0.1% peptone (Difco) solution, pH 7, for 2 min. Duplicate serial dilutions with 0.1% peptone were made and 0.1 ml of appropriate dilutions were spread on the surface of plates containing Baird-Parker medium (Difco) or TSY agar. The plates were then incubated aerobically at 37°C for 48 hr.

Although the ground, fried bacon was not sterile, interference by residual bacteria was not usually observed. However, after prolonged aerobic incubation (over 7 days), and in instances where the growth of *S. aureus* did not occur, moldy samples were occasionally observed.

Extraction of enterotoxin from bacon samples

Each bacon sample (10g) was weighed and homogenized with 20 ml of 0.2M NaCl in a polypropylene centrifuge tube (29 X 104 mm, Dupont Inst., Newtown, CT) with a tissuemizer (Model SDT; Tekmar Co., Cincinnati, OH). The homogenate was centrifuged (27,000 X *g*; RC-2B Centrifuge, Dupont Inst., Newtown, CT), the supernatant decanted, and the precipitate extracted with the same volume of 0.2M NaCl. The supernatants were pooled and the pH adjusted to 4.5 with 0.1N HCl. The resultant precipitate was separated by centrifugation and the supernatant adjusted to 7.2 with 0.1N NaOH. Sodium azide (0.1%) was added as a preservative and the bacon extracts were frozen until analyzed.

Enterotoxin assay

Purified SEA was supplied by R.W. Bennett (Food and Drug Administration, Washington, DC). The lyophilized SEA was rehydrated in phosphate-buffered saline (PBS; 0.07M phosphate, 0.07M NaCl, pH 7.2) and kept frozen until used. The SEA solutions used in the preparation of standard curves were prepared in extracts of uninoculated prefried bacon containing 0.1% sodium azide.

The anti-SEA IgG used in this study was purified from heated serum of a rabbit immunized against SEA. The serum was fractionated by adding 2.5 ml of a saturated ammonium sulfate solution to 2.5 ml of serum while stirring in an ice bath. The supernatant was removed by centrifugation (27,000 x *g*) and the precipitate redissolved in 5 ml of distilled water. The ammonium sulfate fractionation procedure was repeated three times. The precipitate was then dissolved in 0.01M phosphate-buffered saline (pH 7.2), dialyzed against the same buffer to remove ammonium sulfate and then placed in a Sephadex G-200 column (2.2 x 90 cm) to separate IgG from IgM. The fractions were eluded with 0.1M phosphate-saline buffer (pH 7) and the IgG fraction was stored at −10°C.

The igG-coated polystyrene assay tubes were parepared by the procedure described by Stiffler-Rosenberg and Fey (1978). The IgG stock solution was diluted to a concentration of 5 μg/ml in 0.1M $NaHCO_3$ (pH 9.6) and 1 ml was pipetted into polystyrene tubes (10 x 75 mm, Falcon 2038, Cockeysville, MD) and incubated over-

night at room temperature. After removal of the IgG solution, the tubes were washed with PBS and then filled with 1% BSA (bovine serum albumin, fraction V. Sigma Chemical Co., St. Louis, MO) in PBS with 0.1% sodium azide and incubated an additional 2 hr at room temperature, emptied, washed with BSA, drained, and stored at 4°C. The assay tubes were used within 1 wk.

The ^{125}I-labeled SEA was prepared at the New England Enzyme Center, Boston, MA. The iodination was based upon the chloramine T procedure described by Greenwood and Hunter (1963) and modified by Johnson et al., (1971) and Kauffman and Johnson (1975) for staphylococcal enterotoxins. The specific activity of ^{125}I-labeled SEA used in this study was approximately 70 μCi/μg of protein. The titration of freshly prepared ^{125}I-labeled SEA, described by Pober and Silverman (1977), was used to determine the amount of ^{125}I-labeled SEA added to each tube.

With some modifications the radioimmunoassay procedure described by Pober and Silverman (1977) was used in this study. The modifications included the length of incubation of bacon samples in the tubes. Instead of incubating for 4 hr at 37°C, an 18 hr incubation period was used. The sensitivity of the radioimmunoassay used in this study was 1 ng/ml. A standard curve was prepared with each assay and the analysis was carried out in triplicate.

RESULTS

THE RELATIONSHIP between yield, a_w, and the frying time during processing is shown in Figure 2. The bacon lost weight very rapidly during the initial stages of frying, but the yield became minimal when the frying times exceeded 25 sec. The rate of reduction of a_w did not decrease appreciably until the frying time exceeded 30–35 sec.

Although the relationship between the yield, a_w, and the frying time of bacon was nonlinear, the relationships between these parameters were predictable, and therefore fried bacon samples with the desired a_w's could be routinely prepared.

The correlation coefficient between a_w and the major constituents of fried bacon is shown in Table 1. As expected, moisture content had the best correlation with a_w. The fat correlated poorly with a_w, whereas the protein and ash contents correlated well.

Table 1–Correlation coefficients between a_w and the major constituents of precooked bacon

	Correlation coefficient
Protein	–0.9159
Fat	0.5852
NaCl	–0.9188
Moisture	0.9796
Ash	–0.9202

The aerobic growth of *S. aureus* A-100 in bacon is shown in Figure 3. At 37°C (Fig. 3A), the minimal a_w at which staphylococcal growth occurred was 0.84. The narrowness of the growth-no growth range of a_w is indicated by the fact that no growth was observed at a a_w of 0.83.

Since precooked bacon is subject to temperature variation during storage, it was considered important to investigate the interrelationship of temperature on microbial growth and a_w. The aerobic growth of *S. aureus* in precooked bacon at 20°C is shown in Figure 3B. The minimal a_w required to support growth increased from 0.84, obtained at 37°C, to 0.88 at 20°C. The initial cell population declined during incubation when the a_w of the bacon was at or below 0.87, demonstrating that the growth-no growth zone was also very narrow at 20°C. At a_w's of 0.89 and 0.88 a lag period was noted prior to the initiation of growth. The growth rate of *S. aureus* at 20°C was slower than at 37°C since at a a_w of 0.89, and at 20°C, it took 6 days for *S. aureus* to attain a population of 10^8 CFU/g, whereas it only took 1 day to reach 10^9 CFU/g at 37°C. The growth rates at both temperatures generally appeared to be related directly to the a_w of the sample.

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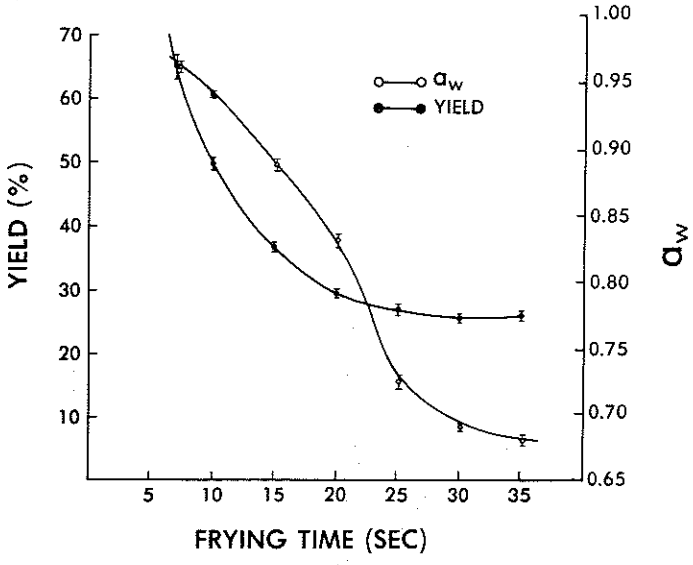

Fig. 2–Relationship between yield, a_w and frying time of precooked bacon: ○–○ a_w (20°C); ●–● yield.

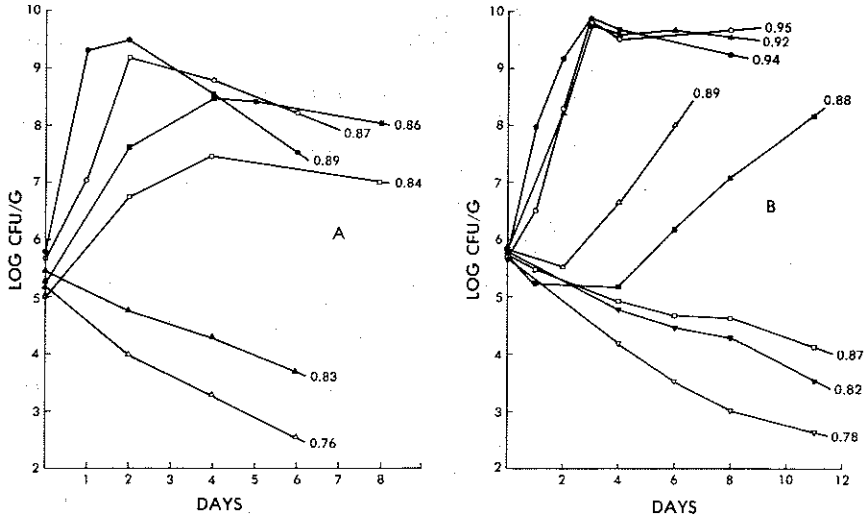

Fig. 3–Aerobic growth of Staphylococcus aureus A-100 in precooked bacon: A, 37°C; B, 20°C.

The higher minimal a_w required for growth at 20°C was reflected in the isotherms for prefried bacon at 20°C and 37°C (Fig. 4). At a_w's greater than 0.74 samples having the same moisture content gave a higher a_w value at 20°C as compared to 37°C. Below 0.74, the isotherms cross and, consequently, this relationship is reversed. Since the water content was reduced by frying these isotherms were essentially derived by desorbtion.

In order to confirm the low minimal a_w required for the growth of *S. aureus* strain A-100 and its rapid growth in precooked bacon in the intermediate moisture range, another strain of *S. aureus* was tested. The aerobic growth of *S. aureus* strain Z-88 in ground precooked bacon at 37°C as a function of a_w is shown in Figure 5. As was observed with strain A-100, strain Z-88 grew at a a_w of 0.84 and very rapidly at 0.88. No growth was observed at a a_w of 0.82.

Commercially, precooked bacon is packed in cans under vacuum. The growth of *S. aureus* A-100 in precooked bacon packaged and stored under anaerobic conditions, is shown in Figure 6. When incubated anaerobically at 37°C (Fig. 6A), the minimal a_w that allowed staphylococcal growth was found to be 0.90, higher than the a_w of 0.84 required for aerobic growth.

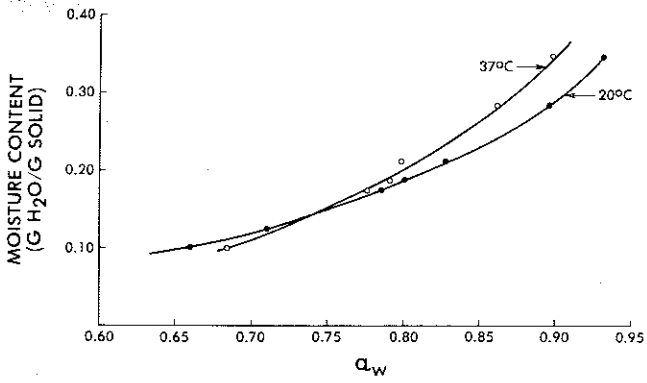

Fig. 4—Isotherms of precooked bacon at 20° C and 37° C.

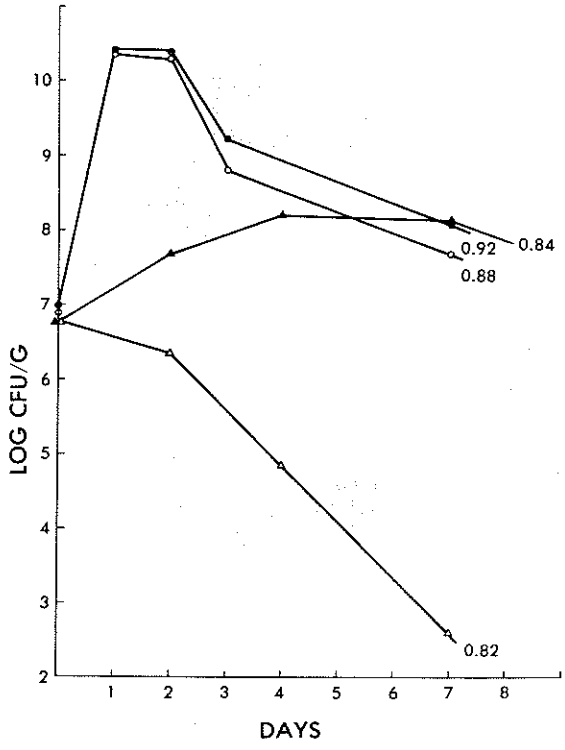

Fig. 5—Aerobic growth of Staphylococcus aurerus Z-88 in precooked bacon at 37° C.

In agreement with the results obtained at 37°C, at 20°C a higher minimal a_w was required to support anaerobic growth than aerobic growth. As shown in Figure 6B, growth, although slight, was observed at a a_w of 0.91 and over a one logarithmic increase occurred at 0.94. At comparable a_w's, the maximal populations achieved anaerobically at either 20 or 37°C were lower than that obtained during aerobic incubation (Fig. 3).

The SEA content of the bacon samples is shown in Table 2. Bacon samples were assayed only at the end of the incubation period. Since the cell population sometimes declined after reaching a maximum, the final cell population may inadequately reflect the extent of the physiological activities of *S. aureus.* Therefore, the maximal as well as the final population of each growth curve are listed. With the exception of the sample incubated anaerobically at 20°C at a a_w of 0.91, SEA was detected in those samples where growth occurred either aerobically or anaerobically. In the sample in which toxin was not detected, the population increase was less than one logarithm. The lowest maximal cell population found in a sample containing SEA (40 ng/g) was 1.2×10^7 CFU/g.

DISCUSSION

THE MINIMAL a_w at which staphylococcal growth is reported to have occurred is not consistent. Scott (1953) reported that the minimal a_w supporting staphylococcal growth in rehydrated milk and soup mix at 35°C was 0.86. Troller and Stinson (1975) indicated that the minimal a_w for the growth of two strains of *S. aureus* in rehydrated potato dough at 37°C was less than 0.88; whereas in shrimp slurry 0.89 was found to be the minimal a_w for growth. In both foods the a_w was adjusted with glycerol. Labuza et al. (1972) reported that growth occurred at a much lower a_w. Using a pork slurry system whose a_w was adjusted by adding glycerol, they reported that a a_w of 0.84 supported the growth of *S. aureus* at 25°C. For intermediate moisture pork dices, though, it was shown that a a_w higher than 0.865 was required for the growth of *S. aureus* at 25°C (Plitman et al., 1973). Glycerol was used as the humectant. The data of Hill (cited by Tatini, 1973) indicated that *S. aureus* could grow in salt pork at a a_w of 0.83 at 35°C. The a_w was adjusted by adding NaCl. Lotter and Leistner (1978), studying the growth of two strains of *S. aureus* in broths whose a_w was adjusted with salts of NaCl, KCl and Na_2SO_4, found that the minimum a_w's that supported growth were between 0.864–0.867 at 30°C and between 0.870–0.887 at 25°C. All of the studies mentioned above were carried out under aerobic conditions. In this study, the minimal a_w supporting aerobic growth of *S. aureus* at 37°C in precooked bacon was 0.84 and increased to a minimal a_w for growth of 0.88 at 20°C. When incubated anaerobically, the minimal a_w's supporting staphylococcal growth increased to 0.90 and 0.91 at 37°C and 20°C, resepctively.

The difference in the minimal a_w reported to be required for the growth of *S. aureus* might have been due to differences in: (1) the nutritional environment for microorganisms; (2) the type of humectant used to adjust a_w; (3) the type and extent of hysteresis and isotherms; and (4) environmental factors such as temperature, pH, and oxygen tension. (Tatini, 1973; Leister and Rodel, 1976).

To determine the minimal a_w for growth in precooked bacon, a defined product to which humectant cannot be added in large concentrations, the main consideration was

given to two major factors—temperature and the presence of oxygen.

Temperature can exert dual effects on microbial growth in the intermediate moisture range. It could affect the water requirement of microorganisms directly through its influence on physiological activities or through its effect on the a_w of foods. As shown in this study, the minimal a_w supporting aerobic staphylococcal growth increased from 0.84 to 0.88 when the incubation temperature decreased from 37° to 20°C. Examining the isotherms obtained at these temperatures (Fig. 4), a bacon sample with a a_w of 0.84 at 37°C had a a_w of 0.87 at 20°C. Therefore, it appears that the difference in isotherms was the major reason for the increased minimal a_w requirement for growth at 20°C. The physiological factor appears to be a minor influence, as reflected in the difference of 0.01 a_w units in the minimal a_w's for growth (0.88 minus 0.87). Obviously this relationship appears valid only above a a_w of 0.74. These results were reversible and are in general agreement with those of Lotter and Leistner (1978) who observed a lower minimal a_w requirement for growth at 30°C (0.867) than at 20°C (0.887). Wolf et al. (1973) studied the isotherms of raw chicken at three temperatures 5°, 45°, and 60°C and reported that for samples with the same water content, the a_w value increased as the temperature increased throughout the range of a_w's studied (0.50–0.80). However, an examination of their isotherms in the higher a_w region (0.60–0.80), indicated that the isotherms might intersect.

As shown in this and other studies, the use of vacuum significantly increased the minimal a_w required for staphylococcal growth. More importantly, no significant physical or chemical changes in the product ordinarily occurs as a result of vacuum packaging, making this a desirable technique for controlling microbial growth.

The minimal a_w supporting anaerobic growth of *S. aureus* in precooked bacon is very close to the value 0.92, reported by Scott (1953) for a biological medium in which the a_w was adjusted by glucose and salt mixtures. Scott (1953) observed slight anaerobic growth at a a_w of 0.92 at 30°C and while not actually observing the limiting a_w for growth, he concluded, that it was unlikely that anaerobic growth would occur at a a_w of 0.90 in his medium.

A problem that might be encountered when using vacuum packaging combined with a_w to control staphylococcal growth in precooked bacon, would be the maintenance of adequate vacuum since a slight leakage of air into the container would greatly enhance the possibility for the subsequent growth of *S. aureus* and the production of enterotoxins. In preliminary experiments to determine our capability to establish anaerobiosis, we used custom built anaerobic hoods (Forsyth Dental Center, Boston, MA) and attempted to degas the samples of bacon in humidity chambers (Fig. 1) by three 10-inch vacuum-nitrogen flush cycles and sealing under nitrogen. Anaerobic indicator strips (BBL, Cockeysville, MD) placed in a humidity chamber to monitor anaerobiosis turned slightly blue after 16 hr, demonstrating the presence of a low oxygen tension inside the chamber. Under these conditions, the minimal a_w supporting growth at 37°C was found to be 0.86 indicating that *S. aureus* grew and produced enterotoxin at low oxygen concentrations. This has also been shown to occur in the author's laboratory for model systems (Werner, 1978) where appreciable concentrations of SEA and SEB toxins were secreted at oxygen concentrations of 2% or less.

Thatcher et al., (1962) had observed the lack of an offensive odor in raw bacon after anaerobic staphylococcal

Table 2—Effect of oxygen, temperature, and a_w on the growth and enterotoxin A secretin by Staphylococcus aureus in precooked bacon

	a_w	Maximum population (CFU/g)	Final population (CFU/g)	SEA (ng/g)	10^8 SEA/CFU[b]
Aerobic incubation					
37°C	0.86	3.0×10^8	4.8×10^7	193	64
	0.84	3.1×10^7	1.8×10^6	19	61
	0.75	1.9×10^5	$< 10^{2}$[a]	0	–
20°C	0.95	6.9×10^9	3.2×10^9	821	12
	0.93	5.8×10^9	3.5×10^9	480	8
	0.89	1.4×10^8	1.4×10^8	26	19
	0.87	5.1×10^5	1.3×10^{4}[a]	0	–
	0.83	5.5×10^5	3.4×10^{3}[a]	0	–
	0.78	4.7×10^5	4.3×10^{2}[a]	0	–
Anaerobic incubation					
37°C	0.94	3.2×10^8	6.4×10^5	109	34
	0.93	3.2×10^8	1.9×10^6	109	34
	0.91	2.8×10^8	4.4×10^6	70	25
	0.90	1.9×10^7	2.6×10^6	38	200
	0.84	1.9×10^5	1.0×10^{4}[a]	0	–
20°C	0.94	1.2×10^7	1.6×10^6	40	333
	0.91	2.0×10^6	2.0×10^6	0	–
	0.89	4.9×10^5	1.4×10^{5}a	0	–

[a] No growth by the inoculum

[b] Maximum population, the time at which the maximum population occurred, can be derived from Fig. 3 and 6.

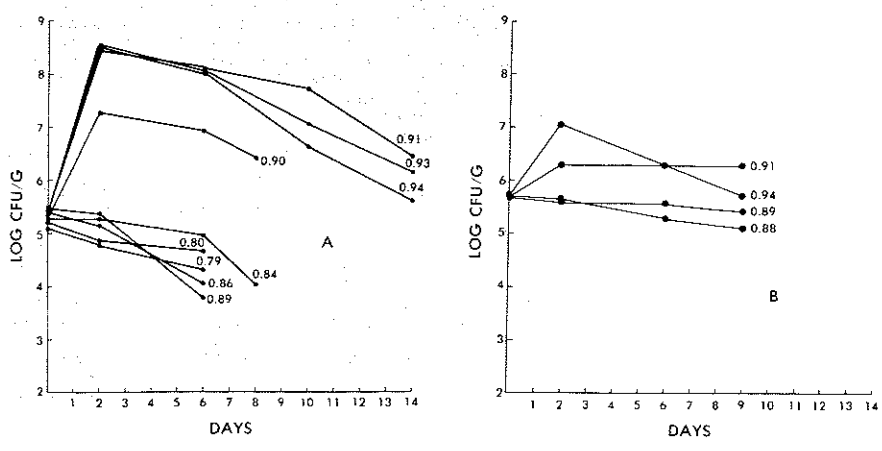

Fig. 6—Anaerobic growth of Staphylococcus aureus A-100 in precooked bacon: A, 37°C; B, 20°C.

growth. In this study no offensive, spoilage odor was noted for prefried bacon stored anaerobically but offensive odors were noted in all samples in which aerobic growth occurred.

The relationship derived in this study between the M/S ratio and the a_w of the precooked bacon is presented in Figure 7. The M/S ratio of 9.0 suggested by Whiting et al. (1965) corresponds to a a_w of 0.91, and which would allow *S. aureus* to grow anaerobically.

Although the M/S ratio can be used as an indirect measurement of a_w for precooked bacon, recent developments in equipment for measuring a_w makes it feasible to directly employ a_w in a quality control program.

Two previous studies also investigated the interrelationship between a_w, the growth of *S. aureus* and enterotoxin production. In both studies humectants were employed and both studies were aerobic. The minimal a_w of 0.84 for SEA production and growth by *S. aureus* in prefried bacon is lower than the 0.93 observed for SEA production in shrimp slurry and potato dough reported by Troller and Stinson (1975). As noted above, the minimal a_w's supporting growth were as low as 0.89 for shrimp and 0.88 for potato. In this study, SEA was detected in every bacon sample where growth occurred except in the one condition where less than one logarithm of growth was observed (from 5×10^5 CFU/g to 2.0×10^6 CFU/g). It is conceivable that if enterotoxin was produced during growth the concentration may have been below the sensitivity of our assay procedure. In agreement with this study, Lotter and Leistner (1978) also found that the minimal a_w for staphylococcal growth and for enterotoxin production was identical. They also showed that the minimal a_w for growth and for SEA production in broth by two strains of *S. aureus*, the a_w being adjusted with salt mixtures, was between 0.864 and 0.867 at 39°C and 0.870–0.897 at 25°C.

Enterotoxin production by *S. aureus* in prefried bacon was considerably less effective than in the model systems of Troller and Stinson (1975) and Lotter and Leistner (1978), being, in a number of instances less than 100 ng (Table 2). For this reason the radioimmunoassay, capable of detecting enterotoxin concentrations of 1 ng/ml of extract, was employed. Moreover, the use of purified IgG to replace crude antisera in coating the polystyrene tubes and a precoating procedure (Pober and Silverman, 1977) reduced nonspecific interference during assay. The use of a sensitive assay also avoided losses due to separation and concentration procedures (Niskanen and Lindroth, 1976).

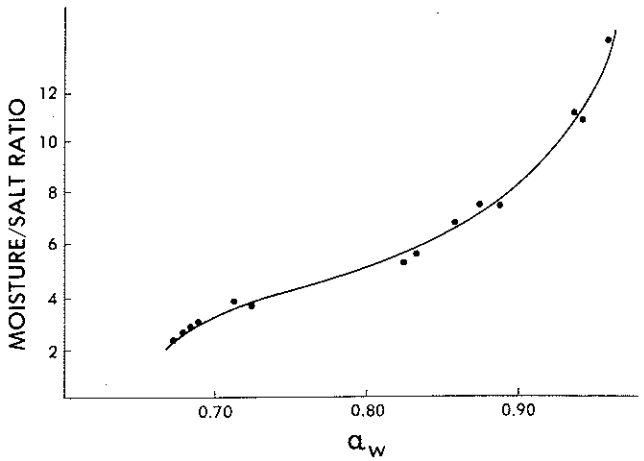

Fig. 7—Relationship between the moisture/salt ratio and the a_w (20° C) of precooked bacon.

The extent to which oxygen, temperature, and a_w interact to influence enterotoxin secretion is not apparent. Although, at comparable a_w's, higher levels of enterotoxin were produced aerobically and also at 37°C, enterotoxin secretion per unit number of cells (maximal population, Table 2) was not decreased by anaerobiosis or by lower temperatures. This indicates that even under sub-optimal growth conditions, if *S. aureus* can initiate and sustain growth, then the cell can efficiently synthesize and secrete toxin. The fact that secretion and the growth of *S. aureus* are interrelated is also supported by the previous studies for SEB (Dietrich et al., 1972) and for SEA (Czop and Bergdoll, 1974; Carpenter and Silverman, 1976), whereby these enterotoxins were produced during all phases of growth.

Although precooked bacon is subject to further heat treatment before serving, the cooking time may be minimal and any enterotoxin present may, due to its thermal stability, still be biologically active. Of course, losses in enterotoxin would occur due to the subsequent removal of fat and moisture during frying. The lowest level of SEA detected in the bacon samples in this study was 19 ng/g. At this concentration, a person eating approximately 60g of bacon might become ill, since the emetic dose of SEA for a human is considered to be about 1 μg (Bergdoll, 1970). Therefore, to avoid a public health hazard, it is best for the processors to observe hygienic procedures and follow rigid quality control programs to prevent the entrance into or proliferation of *S. aureus* in their product.

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